RECENT DEVELOPMENTS IN THE STUDY OF TUBERCULOSIS OF INTEREST TO PHYSICIANS*

Wm. Charles White

The tuberculous process in man and animals involves the living together of two independent organisms, the tubercle bacillus and the monocyte ameboid cell. Each of these has its own living chemistry. It would seem however as if they had certain common factors in their search for food supplies. Otherwise their close association in what we know as the tubercle would probably not be so universally found in the early history of the development of tubercles.

Under certain conditions with which we are at the present time little familiar, the living together of the two component parts of the tubercle may change in one of two directions, either the bacillus does not find a food supply adequate for its life and disappears from the scene, or it rapidly multiplies within the monocyte robbing the latter of its means of subsistence so that it dies and collections of these monocytes known as the tubercle coalesce and undergo a process known as caseation, in which substance the tubercle bacilli almost cease to exist as acid-fast rods but probably exist in some other form with a different chemistry. This caseous material then undergoes softening and usually ruptures from its surrounding environment. At this stage the acid-fast form of the organism begins again to appear. If the rupture takes place to the outside of the body and the acid-fast organism is fortunate enough to find new hosts then it goes through the same cycle. If the rupture is into some cavity or vessel within the body then the organism goes through the same process that has just been described in another part of the same body. This conjugate life of these two living cells along with the de-

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** Delivered before The New York Academy of Medicine, March 10, 1933, in the Friday Afternoon Lecture Series.*

velopment of new methods of study in the laboratory has opened the door for a new attack on the tuberculosis problem.

I might interject here that the tuberculosis problem in all its ramifications is the most expensive disease problem with which man has to deal. The individual case is the most trying with which the practitioner of medicine has to deal and with our present methods it is a long discouraging problem both for the patient and the physician.

Taking now the two living factors that go into the formation of the tubercle I would first bring your attention to what we know of the monocyte or host cell and what methods are available for studying its life history and then describe to you the modern attempts in chemistry and biology to study the tubercle bacillus or infecting parasite, and thirdly, describe the influence of these studies on modern thought in connection with patients suffering with this disease. Before doing this I would like to state that the Director of The New York Academy of Medicine, Dr. Linsly Williams, has been an interested participant in these studies from the beginning.

The monocyte cell is an independent ameboid cell and one of the component cells of all vertebrate animal bodies. Within the body it has three possible forms of motion:

1. Inherent power of moving by ameboid action from place to place.
2. Movement by the blood stream.
3. Movement by lymphatic flow.

In the development of vertebrates the monocyte appears first by differentiation of cells from the mesoderm. Part of the mesodermal cells become angioblasts, giving rise to the endothelial lining of vessels and part of them remain outside this lining to develop into what is known as fixed connective tissue cells and these are apparently able to develop into monocytes or clasmatocytes or fibroblasts under the influence of certain environmental stimuli. In those animals which have been carefully studied, however, their main place of origin, in the fully grown animal, is in the connective tissues. They also arise in the lymph glands which, as you know, are a frequent site of tuberculosis. Cells very similar to them are produced also in the pulp of the spleen and appear in greater abundance in the splenic vein which flows directly into the portal vein going to the liver.

Amebas in nature tend to round up and rest at certain periods of their life history. Their food supply is largely bacteria. Under certain conditions they develop what is known as multi-nucleosis. That is their nuclei divide without the whole cell body dividing. In this condition they are known to us as giant cells. Monocytes in the body tend to form a similar type of giant cell in the tuberculous nodule. Their specific functions in the body are yet undecided problems. This much, however, seems certain—that they have in some way to do with the utilization of certain classes of fats and it is in this point of their function that we will probably find their chemical relation to the tubercle bacillus, which under proper conditions can grow so abundantly within the body of these cells. One fact stands out—that in neither the tubercle bacillus nor in the monocyte is there evidence that the sterols, such as cholesterol, are an important step in whatever their chemical function in connection with the fats may be.

One of the great difficulties in studying the living chemistry of the monocytes is the lack of a method to obtain them in pure culture, although it is possible that the recent method described by Rous and Beard in which they load the Kupffer cells with iron and then withdraw them from their normal environment in the liver by an electric magnet may bridge this gap in our technique.

An easier field of study from the standpoint of living chemistry is offered by the tubercle bacillus. This can be obtained in pure culture and even a pure culture developing from a single cell. This culture can be produced in great quantities on a constant medium, it can be extracted by various solvents and the extracts from these solvents be produced in sometimes pure and crystalline form and the effects of these pure substances tried on the living normal and tuberculous animal. One of the difficult problems however in this procedure is that tubercle bacilli are members of a vast family known as the acid-fast family and the number of strains in this family, each with its peculiar chemistry, increases so rapidly that the work of analysis seems almost endless. This much however can be said, that whenever any member of this family is introduced into the animal body it always, whether it produces disease or not, has some relation to the monocyte cell. If it be a virulent strain it will rapidly multiply in these cells as the beginning of the disease process, and if it be avirulent it soon ceases to exist in the body.

For the past seven years a group of students, many of them here in New York and members of the Academy, have been interested in the study of the chemical fractions that can be isolated from the different strains of tubercle bacilli and the influence of these on the animal body. I may mention among these Dr. R. J. Anderson, Dr. Sabin and her associates, Dr. Heidelberger and Dr. Kendall, Dr. Long and Dr. Seibert, Dr. Richardson, Dr. Loebel, Dr. Shorr and Dr. Kahn.

The substances which have been isolated belong to the proteins, the phospho-lipins, the waxes and the carbohydrates. For the isolation of these substances five strains of acid-fast bacteria have been used. They have all been grown upon a common medium and a careful comparison drawn between the quantities of the substances which each different strain produces. These bacillary strains are human, avian, and bovine tubercle bacilli which are virulent, and two avirulent strains.

Such substances as have been so far isolated are common to all strains but there are very large and significant differences in the quantities of a number of these isolated substances that each strain of organism produces. This is especially striking in the carbohydrate and phospho-lipin fractions and one may say that as the phospho-lipin increases the carbohydrate fractions decrease and vice versa. Two great objectives are sought by the analysis of these bacterial strains. First, there is the hope that we may find a law in synthesis sharply demarking the virulent strains from the avirulent strains and that by knowing the law which contributes to virulence in a bacterium we may, by introducing some substances into the body, break the chain by which a bacillus may continue to grow and kill an animal. This breaking of the chain may be conceived of in simpler terms, as, for example, starving the bacillus or enriching the powers of utilization of food supplies by the monocyte. You will readily realize that this is quite different from the old thought of chemotherapy as developed by Ehrlich in which a dose of a drug fatal to the invading parasite but harmless for the body cell was the goal. This type of therapy is exemplified by the use of arsenic and antimony for trypanosomes and treponemata and by quinine for the malarial parasite. Such a method is probably not applicable in tuberculosis. In trypanosomiasis and spirochætosis the invading organisms have their life quite outside of the cells of the body. In malaria there is an extra-cellular cycle of the parasite during which its death is brought about, but the intracellular life history of the tubercle bacillus within the monocyte is quite a different story. Secondly, by knowing the physiological effects of introducing the fractions of the bacillus into the body it seems possible that other substances may be developed of known chemical activity which may be introduced into the body and stop the destructive processes of the life of the parasite—such destructive processes for example as caseation and softening of the large tuberculous aggregation of cells.

There are now known to be several proteins in the tubercle bacillus family. Some of these, when introduced into the normal animal body in their undenatured form, produce results similar to those produced by all natural foreign proteins. This has been beautifully shown by Dr. Sabin, Dr. Seibert, and others. The animal for instance becomes both anaphylactic and skin sensitive to the reinoculation of the same protein at a later time. This protein is common to all the acid-fast family and will undoubtedly have some definite bearing in the final use of our diagnostic test known as the tuberculin test.

A curious state of affairs has been found in rural Minnesota where the skin of children living upon farms has been more sensitive to this undenatured protein from the timothy grass bacillus than that from the tubercle bacillus.

After a careful study of the literature of the early experiments of Koch with tuberculin on which he based his proclamation of a cure, it seems to me the story is something like this. When he used his first extracts of tubercle bacillus on guinea pigs and got such favorable results he probably was using the unheated extracts and therefore was introducing protein with definite antigenic power, but when he was forced to use his extracts on human beings he found it necessary to boil his extracts and thus spoiled the antigenic power of his proteins and from that time onward, tuberculin has been a boiled product and devoid of antigenic properties. It seems possible that a new era may be approaching in which experiments will be carried on with undenatured proteins of the bacillus which possess this antigenic power and probably a reconstruction of our view about the value of the extracts of the tubercle bacilli in curative medicine. In other words I think Koch was unfamiliar as we all have been with the fact that heating the unchanged protein of the tubercle bacillus destroys its antigenic value and possibly its curative value.

The general result of our studies on the pure proteins of the acid-fast family has been the production of a substance for the testing of skin-sensitiveness in the tuberculous which in the first place can be quantitatively diluted and it is our hope that secondly it can be so diluted in solution that it will give a specific skin test for the presence in the body of virulent bacilli and perhaps we may even hope a specific test for a given strain of bacillus. One sees in the modern use of tuberculin, a procedure quite contrary to all our other good biological tests in that we proceed by increasing the amount of tuberculin until we get the maximum number of reactors rather than a procedure of diminishing the quantity until we get an evidence of specificity which would be of real diagnostic value.

The physiological action of this undenatured protein in the normal animal and in the tuberculous animal has been carefully worked out by Dr. Sabin and her associates. One of the specific cellular responses in the normal animal to the undenatured protein has been found in the study of Dr. Franklin Miller who has been able to work out the differentiation and production of one of the rare cells of the body known as the plasma cell. The influence of this protein on the temperature of the body, on blood pressure, on injury to endothelial cells and the central nervous system has been carefully studied by Dr. Sabin and Dr. M. I. Smith. The work in this field is still progressing very rapidly under Dr. Seibert looking towards the determination of the size and chemical structure of the molecule responsible for each of these physiological responses.

With our present knowledge it may be said that, in general practice in testing with tuberculin in man and animals, a positive tuberculin test is the evidence of some member of the acid-fast family growing in the body. It is not proof that this is a virulent member of the group and our studies will not be complete until we have a diagnostic substance for testing that will answer this question.

If we increase the quantity of the undenatured protein of the timothy bacillus we obtain the same reaction as that obtained from a very minute quantity of the protein obtained in the same way from the bovine bacillus. The protein obtained in our studies from the bovine strain is by far the most potent for this type of reaction. That is it requires less of it than of the protein from any of the other strains. We have no satisfactory explanation to offer of this fact.

The Phosphatide Fraction

The fatty fractions of the tubercle bacillus have been separated by their solvents into several well-known groups such as, for example, an acetone soluble fat, a phosphatide, and a chloroform soluble wax. Early in these studies it was found that the so-called phosphatide, a phosphorus containing lipin combined with a polysaccharide, had the property, when introduced into a normal animal, of stimulating the multiplication of the monocyte cell thus probably bringing this fraction into a close chemical relation with the chemical function of this cell in the body. This phosphatide is taken up by the monocyte cell and digested slowly. The monocyte cells containing the phosphatide pack together much as they do in tuberculosis and if the dose be large enough they coalesce and undergo caseation. This change to caseation is enhanced by the introduction at the same time of the undenatured protein fraction referred to above. This property which the phosphatide possesses has been found by further analysis to belong to an optically active fraction of the phosphatide named by Anderson "phthioic acid". It is a common substance in all strains. The production of this material by the growth of the living bacillus outside of the body and its action upon cells of the body after death of the bacillus is undoubtedly related in causation to two of the prominent processes present in the natural disease. The work of providing substances which may interfere with these processes of the massing of monocyte cells and coalescence and caseous change, is surrounded with so many difficulties that up to the present very little progress has been made. As new methods appear and more knowledge accumulates it seems possible that a door will be opened to offset one or other of these most destructive chemical actions which accompany tuberculosis.

It is a remarkable thing that this family of bacilli should have the power to build from a simple medium such an amount of phospho-lipin in combination with such a complex polysaccharide. The importance of phospho-lipin in all living cell activity has of late years been growing very rapidly. One may say that wherever life occurs these phospho-lipin sugar combinations stand out as an integral necessity in life activity. One is presented in the tubercle bacillus growth on a simple medium with an opportunity of studying the mechanism by which synthesis of phospholipins occurs, and the intermediate products which are established in the conversion of glucose into fatty acids and their later combinations with glycerol and phosphorus to form some higher straight chain compounds of carbon. Anderson's masterly work in demonstrating the power of this family to produce a fatty acid of twenty-six carbon atoms in chain is among the great achievements of modern bacterial chemistry. In this phosphatide fraction he has already shown the presence of butyric acid with four carbon atoms, palmitic acid with 16 carbon atoms, and stearic, linoleic, and linolenic acids with 18 carbon atoms. When one attempts to correlate this with the function thought to occur in the synthesis of fatty acids in the perfused liver from salts of pyruvic and lactic acid and the probability of further synthesis in the liver and lung, synthetic functions that may be in part inherent qualities of the monocyte cell, it is possible to let the imagination run to the belief that there is close relation in the synthetic chemistry of these two living cells and that when we understand it we may be a long way on our journey towards a cure of tuberculosis. But this is no place for imagination and I must recount only the facts as we now know them.

Waxes

The waxes which have been isolated from the bacilli produce lesions of so complex a character, when introduced into the normal animal, that little can be said at this time of their function. The waxes have, as you know, in the past been variously considered as the most important part of the bacillus. The literature is full of statements about the "waxy capsule" but so far as our studies are concerned, there is not the slightest evidence that the wax forms a capsule rather than being part of the body of the bacillus. The wax from the tubercle bacillus is a higher alcohol. There is no evidence that waxes in nature take part in the vital processes of the living organism. They are rather protective in character against the action of water and other penetrating and degrading agents. When injected into the body this wax induces the multiplication of connective tissue cells which fuse to make foreign body giant cells around the particles. Thus, with reference to the lesions of the disease, this reaction may be considered as non-specific.

Sugars

The significance of sugars in combination with proteins and lipins in living chemistry has tremendously increased in the last few years largely through the studies of Avery, Heidelberger, and Laidlaw and Dudley. That there should be so vast a difference in the biological activity caused by the spatial difference of one hydroxyl group in a molecule, as shown by Avery and Goebel, and that there should be a difference of 10 times in the physiological activity of codeine and pseudo-codeine with only the spatial difference of one hydroxyl group in the molecule is so significant of the importance of these simple chemical differences in physiology that one has to restrain oneself in picturing the future of accurate chemical, biological knowledge.

And yet Emil Fischer, the father of modern carbohydrate chemistry, did not hesitate in his address before the German Chemical Society in 1890 to let his imagination run in these words:

"Next to the albumens the natural carbohydrates form the chief food stuff for the animal kingdom, especially for graminivorous animals, and a large number of valuable observations have been made concerning the processes they undergo in the animal body. Might it not be possible to substitute some of the artificial sugars for these natural carbohydrates, and what would be the result? Mannose so closely related to grape sugar, and so easily fermented by yeast, might very probably form a good foodstuff, even for the more highly organized animal; and yet the slight change of substance might cause corresponding changes in the vital processes. If mannose be taken as food, will the liver produce a new glycogen, and the mammalian gland a substitute for milk sugar; and will this sugar be oxidized in the body of the diabetic? The changes in the animal organism could not but be still more decided, if one could succeed in feeding the animal body with a pentose or a heptose, or the easily fermentable nonose. One would then probably find that blood and tissues would modify their functions, that the pig and the goose would produce a changed fat, the bee a changed wax. Indeed the experiment might be carried still farther. The assimilating plant prepares from sugar not only the more complicated carbohydrates and the fats, but also, with the help of inorganic nitrogenous compounds, the albuminoids. Certain classes of bacilli have the same power. Now, if it were possible to feed the assimilating plant or these bacilli with a differently constituted sugar, they might possibly be forced to form a changed albumen. May we not then expect that the changed building material will lead to a changed architecture? We should thus gain a chemical influence on the formation of the organism which would necessarily lead to the most extraordinary phenomena, to changes of form far exceeding all that has been reached by crossbreeding, etc. Since the fundamental experiments of Wholer and Frerichs, physiological chemists have incorporated hundreds of organic substances with the animal body, seeking the products in the urine; but they, almost exclusively, made use of substances having no likeness to natural foodstuffs. The use of the new series of sugars offers a wide field of action to the physiologist, and may be attended by results far more extraordinary. Biology here stands before a problem, which has never yet been set, nay, which could not have been set until chemistry had prepared the material for the experiment."

Early in the history of these studies it was shown in England and in America that the crude polysaccharides of the tubercle bacillus family had immense power of causing a precipitate when brought into contact with the serum of animals injected with the whole dead bacillus. I was able to show, by using one of Anderson's polysaccharides found in union with the phosphatide fraction, that there was a profound influence on the temperature and blood pressure and in the distribution of the lipin substances in the cortex of the adrenal glands, when this polysaccharide was injected into the tuberculous animal. The relation of the adrenal cortex to blood pressure and temperature has been emphasized by Cannon and by those working with extracts of this part of the gland. Dr. Sabin has made a careful study of the blood changes produced in the normal animal by use of the same substance. It is very destructive to polynuclear leukocytes. While we know it is such a poison we have so far been unable to demonstrate it in the serum of the tuberculous animal. We are sure that it must be there in some masked form. We feel sure also that it has some definite relation to the daily temperature curves of the very sick animal. Of late months there has been a growing belief that there is a daily death of a generation of tubercle bacilli in the advancing tuberculous animal coupled with the release by lysis of this poisonous substance which may be the underlying cause of the febrile cycle and that if it were possible to demonstrate its presence and cause its destruction there might be one method of controlling one of the most distressing symptoms of tuberculosis. This polysaccharide is a very stable substance requiring boiling with acid to break it up. When broken up it yields such simple sugars as mannose, inosite and d-arabinose. These simple sugars when injected into an animal have no biological significance nor do they have any influence, when put in the medium in the test tube, on the growth of the bacillus. It is only when they are united with phosphorus and nitrogen in a stable polysaccharide that their poisonous effect is produced. This poisonous polysaccharide has no influence on the normal animal and when injected appears in the urine unchanged so that one is forced to the conclusion that the tuberculous animal develops a mechanism which operates in conjunction with the polysaccharide to bring about the death of animals in a few hours.

To carry farther a report of these studies which have been carried out under the Committee on Medical Research of the National Tuberculosis Association would lead us into the intricacies of chemistry and biology that would not be suitable for those not intimately and daily in association with these sciences. I trust in presenting them to you and attempting some correlation of their findings that I may have indicated to you the trend of modern research, using new implements of study, and aroused in you the hope that is in us that we may, by pursuing them, achieve the knowledge which will put in the hands of the physicians implements effective in the control of the disease in the individual patient and enable us, as a people, to control the disease which takes so great a toll of our young adult life and of those animals which we use for the maintenance of our life.